agronomy

MDPI

Article

Enhancing Nutritional and Functional Properties of Broccoli Leaves Through Selenium Biofortification: Potential for Sustainable Agriculture and Bioactive Compound Valorization

Melina Soledad Buttarelli [1,2], Gabriel Céccoli [1,*], Betiana Soledad Trod [1,3], María Micaela Stoffel [3], Marianela Simonutti [1], Carlos Alberto Bouzo [1,3], Valeria Rosana Turowski [4], Adrián Alejandro Perez [5], Mercè Llugany [6], María-Jesús Sánchez-Martín [7], Lucas Damián Daurelio [1,3], María Gabriela Guevara [4,*] and Fernando Felipe Muñoz [3,*]

1 Laboratorio de Investigaciones en Fisiología y Biología Molecular Vegetal (LIFiBVe), Instituto de Ciencias Agropecuarias del Litoral (ICiAgro Litoral), Univerdidad Nacional del Litoral, CONICET, FCA, Kreder 2805, Esperanza 3080, Argentina; melinabuttarelli@yahoo.com.ar (M.S.B.); betianatrod@hotmail.com (B.S.T.); simonuttim@gmail.com (M.S.); cbouzo1160@gmail.com (C.A.B.); lucasdaurelio@gmail.com (L.D.D.)
2 Agroecosistemas I (Nodo de Integración I), Departamento de Biología Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, Esperanza 3080, Argentina
3 Cátedra de Fisiología Vegetal, Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, Esperanza 3080, Argentina; stoffelmariamicaela@gmail.com
4 Instituto de Investigaciones Biológicas (IIB), Universidad Nacional de Mar del Plata, CONICET, Funes 3250, Mar del Plata 7600, Argentina; vturowski@gmail.com
5 Área de Biocoloides y Nanotecnología, Instituto de Tecnología de Alimentos, Facultad de Ingeniería Química, Universidad Nacional del Litoral, 1 de Mayo 3250, Santa Fe 3000, Argentina; adrianperezrubin@hotmail.com
6 Plant Physiology Group (BABVE), Faculty of Biosciences, Universitat Autonòma de Barcelona, 08193 Bellaterra, Spain; merce.llugany@uab.cat
7 GTS Research Group, Department of Chemistry, Faculty of Science, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; mariajesus.sanchez@uab.cat
* Correspondence: gabrielcnbj@yahoo.com.ar (G.C.); gguevara@mdp.edu.ar (M.G.G.); fermunoz81@gmail.com (F.F.M.)

Academic Editor: Alessandra Carrubba

Received: 20 December 2024
Revised: 30 January 2025
Accepted: 30 January 2025
Published: 31 January 2025

Citation: Buttarelli, M.S.; Céccoli, G.; Trod, B.S.; Stoffel, M.M.; Simonutti, M.; Bouzo, C.A.; Turowski, V.R.; Perez, A.A.; Llugany, M.; Sánchez-Martín, M.-J.; et al. Enhancing Nutritional and Functional Properties of Broccoli Leaves Through Selenium Biofortification: Potential for Sustainable Agriculture and Bioactive Compound Valorization. *Agronomy* **2025**, *15*, 389. https://doi.org/10.3390/agronomy15020389

Abstract: Selenium (Se) biofortification is a promising agronomic strategy to enhance the dietary intake of this essential micronutrient while simultaneously adding value to agricultural by-products like *Brassica oleracea* L. var. italica leaves. This study evaluated the effects of foliar Se biofortification on a fresh market broccoli cultivar ('Belstar') using selenite and selenate (1 and 2 mM). Growth performance, biochemical properties, nutraceutical quality, and phytohormone profiles of broccoli leaves were analyzed, highlighting their potential as functional by-products. Multivariate analysis revealed that 2 mM selenite application was the most effective treatment, significantly improving several parameters. Selenium biofortification with 2 mM selenite increased essential nutrient content, including Se, Ca, S, Fe, Mn, Mg, and Mo. It also enhanced the soluble protein content (+2.2-fold), phenolic compounds (+1.5-fold), and total antioxidant capacity (+1.4-fold) compared to control plants. In this sense, the nutraceutical quality of broccoli leaves was markedly improved, supporting their use as a source of bioactive ingredients. Additionally, to assess practical applications, water-extracted Se-enriched broccoli leaves demonstrated antifungal activity against the plant pathogen *Fusarium solani*, attributed to Se-induced alterations in phytohormone profiles. These findings suggest that Se-biofortified broccoli leaves can serve as a sustainable source of essential nutrients and bioactive compounds for the food industry. Furthermore, their antifungal properties position them as potential eco-friendly biopesticides to combat plant pathogenic fungi, thereby promoting sustainable agriculture.

Keywords: brassicaceae; selenate; selenite; by-products; *Fusarium solani*

1. Introduction

Selenium (Se) is an essential micronutrient for animals and humans and is involved in the function of the catalytic center of different selenoproteins [1]. Several studies have reported that Se has antioxidant effects [2,3], which can be related to immune functions and anticancer properties [4]. It is a natural element found worldwide in soils [5]. However, Se deficiency in the population occurs when the concentration of this mineral in the soil is low, and, consequently, the plants exhibit reduced accumulation. It is estimated that over one billion people worldwide suffer from selenium deficiency [6]. Thus, vegetable crops enriched with Se from various species are receiving significant research interest [5,7–9]. In this particular instance, Se biofortification in brassica species such as kohlrabi (*Brassica oleracea* var. gongylodes), white cabbage (*Brassica oleracea* L. var. capitata), red cabbage (*Brassica oleracea* var. capitata f. rubra), savoy cabbage (*Brassica oleracea* L. var. sabauda), cauliflower (*Brassica oleracea* var. botrytis), and broccoli (*Brassica oleracea* L. var. italica) is an effective strategy for increasing the intake of Se by humans without exceeding the maximum tolerable intake (400 μg Se day^{-1}) [10–14]. Therefore, some studies have reported the foliar application of Se in broccoli plants to achieve commercial production of Se-enriched broccoli under field conditions [13,15–17]. In a previous study, it was demonstrated that the foliar application of Se to broccoli plants is an effective and efficient strategy for obtaining plant products (broccoli heads) that could be good sources of Se and should not represent a risk of toxicity for human consumption [13]. We also demonstrate that the level of Se was greater in leaves than in the head after Se application, indicating the potential value of this agricultural biomass [13].

Broccoli by-products, particularly leaves, represent an underutilized resource with significant potential for valorization in sustainable agricultural and industrial practices. Broccoli heads constitute less than 25% of the plant's total aboveground biomass, leaving substantial crop residues such as leaves and stems [18–21]. These by-products pose significant challenges to global agricultural efficiency, as they represent considerable waste with potential negative environmental impacts [19,22]. However, reducing agricultural biomass waste is essential for fostering sustainability and supporting the transition to a circular economy. The recovery and valorization of these residues offer promising solutions to mitigate waste [20], with recent advancements emphasizing their transformation into valuable products such as biofuels, bioplastics, and functional materials, thereby reducing dependence on finite resources and mitigating environmental pollution [23]. Broccoli leaves, for instance, have been identified as rich sources of bioactive compounds, including glucosinolates, phenolics, and antioxidants, which can be utilized in functional food formulations [19,20,24]. Additionally, these by-products have shown promise as plant growth promoters and raw materials for bio-based antimicrobials targeting plant pathogens [21–24]. The development of a biomass economy has further highlighted the potential to convert agricultural by-products into economically viable solutions, such as energy production, bioactive chemicals, and biodegradable materials, reducing greenhouse gas emissions and minimizing landfilling [25–27]. Innovative approaches such as bioprocessing and biofortification further enhance the value of broccoli residues, contributing to more sustainable and circular agricultural systems while creating new economic opportunities [28–30].

The present study aims to evaluate the impact of selenium biofortification, focusing on the application of two different Se species (selenite and selenate) at concentrations of 1 mM and 2 mM, as a strategy to enhance both the nutritional value and functional properties of broccoli leaves. Selenium biofortification was investigated for its ability to increase Se content, improve antioxidant capacity, and elevate phenolic compounds and soluble protein levels, thereby enhancing the nutraceutical quality of these by-products. The study also explores the antifungal activity of Se-enriched broccoli leaf extracts against *Fusarium solani*,

attributed to a Se-induced modulation of phytohormones, including methyl jasmonate and salicylic acid. By addressing global Se deficiencies and promoting environmental sustainability through the valorization of agricultural residues, this work highlights the dual benefits of Se biofortification for improving human health and supporting sustainable agricultural practices.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Broccoli seeds [*Brassica oleracea* var. *italica* 'Belstar' F1 (Bejo Zaden B.V.)] were sown in multicell trays and then grown in a greenhouse located at the Experimental Field for Intensive and Forestry Crops, Faculty of Agricultural Sciences, Universidad Nacional del Litoral (Esperanza, Santa Fe, Argentina), during the autumn–winter season of 2022. After four weeks of growth, seedlings of the same size were selected to improve plant uniformity. The seedlings were transferred to 15 L pots with a substrate composed of GrowMix® Multipro (Terrafertil, Buenos Aires, Argentina) and were irrigated periodically with 50% Hoagland solution. Pots were placed 0.6 m from each other in rows spaced at 0.7 m, obtaining a plant density of 2.4 plants m^{-2}. Plants were placed on three subplots (49 plants per subplot), where a completely randomized plot design was applied. In each subplot, edge effects were considered. Broccoli plants were grown for 90 days in a greenhouse under semi-controlled conditions with 76% relative humidity, an average temperature of 27/16 °C (day/night), and a PAR solar radiation of 800 µmol m^{-2} s^{-1}.

2.2. Foliar Application of Selenium

The foliar application of Se was carried out following the methods of Muñoz et al. [13] and Trod et al. [31]. Briefly, at the beginning of head formation (main stage 41 of plant development of harvestable vegetative parts, according to the BBCH phenological scale) [32], each plant was sprayed with approximately 10 mL of a solution containing 0, 0.5, or 1 mM of selenate or selenite (Na_2SeO_4, Na_2SeO_3; Sigma-Aldrich, Berlin, Germany). This stage, equivalent to 68 days after transplanting (DAT), was characterized by a growing tip width greater than 1 cm. During spraying, 0.1% Rizospray Extremo® (Rizobacter, Rosario, Argentina) was used as an adjuvant to facilitate foliar penetration of Se. The application of Se was repeated after 10 days (main stage 43) to reach a final concentration applied of 0, 1, and 2 mM. Distilled water with the adjuvant was utilized for treatments without Se supply (control). Control plants were separated physically with plastic foil to avoid cross-contamination during spraying. For each treatment, three plants were employed (n = 3), considering all the parameters measured and determined in this study [13].

Plants were harvested 90 DAT at commercial maturity (when the heads reached their maximum size and presented green and compact flower buds) and were immediately transported to the laboratory in a cold storage cabinet on ice to preserve their freshness. The different analyses on the heads (fresh weight, firmness, and diameter) were performed within 3 h postharvest.

2.3. Morphological and Physiological Parameters

Plant height (PH), plant leaf area (LA), and the plant chlorophyll index (CI) were measured at 78, 85, and 90 DAT. These time points were selected considering the stages of broccoli head development. At 78 DAT, 10 days after the first Se application, parameters were measured to assess initial effects. At 85 DAT, when the heads reached approximately half of their commercial size, additional measurements were taken. Finally, at 90 DAT, when the heads reached optimal commercial quality, the last set of parameters was measured.

These stages were based on protocols previously described by Muñoz et al. [13] and Trod et al. [31].

Plant height was measured using a ruler. Three randomly selected plants per treatment were measured from the ground level to the apical meristem. After that, Plant Relatives Elongation Rate (PRER) was calculated. To estimate the PRER, height measurements were transformed into natural logarithms and plotted against the DAT. A linear regression fitting in the interval comprising the days of measurements was performed, and the slopes were PRER estimates [33].

The estimation of LA per plant was performed following the allometric Equation (1) described by Céccoli et al. [33]:

$$\boldsymbol{LA} = (\boldsymbol{Lx}\ \boldsymbol{W} * \boldsymbol{F}) * \boldsymbol{n} \quad (1)$$

where L and W are the length and width of the central leaflet of each leaf (cm), respectively; F is the allometric factor (0.615) calculated from leaf samples (n = 100); and n is the number of leaves per plant.

The CI was measured with a SPAD-502 chlorophyll meter (Minolta Camera Company, Tokio, Japan).

At harvest, the main broccoli heads were cut off, leaving a 5 cm long stem. The heads, leaves, and stems were weighed, and fresh weights were recorded. Then, the broccoli tissues were dried at 65 °C until they reached constant weight to calculate the dry weight.

Surface firmness measurements on each head were conducted at three different positions using a digital Turoni durometer with a spherical tip (model 53215TT; T.R. Turoni®, Forli, Italy). The average values obtained were expressed in shore firmness units.

Head diameter measurements were taken with a digital caliper on two opposite sides in the equatorial zone on each head. The average values were reported in millimeters.

2.4. Biochemical Parameters

Briefly, the leaf from the seventh internode of three plants per treatment (including control plants without foliar selenium application) was collected and used in triplicate for all biochemical parameters measured (n = 3). After leaf collection, each leaf was individually handled for biochemical quantification. The detailed manipulation of the leaves is explained in Sections 2.4.1–2.4.6.

2.4.1. Soluble Protein Content

The extract was prepared by grinding 0.3 g of frozen broccoli leaves with liquid nitrogen in a chilled grinder, followed by the addition of 1 mL of 50 mM sodium phosphate buffer (pH 7). The sample was recovered and centrifuged at 16,090× *g* for 20 min at 4 °C (Thermo Scientific Sorvall ST16R, Fisher Scientific, Waltham, MA, USA). The supernatant was placed in 2 mL Eppendorf tubes, and the precipitate was discarded. The soluble protein content in the supernatant was determined by the Bradford method [34]. The results were expressed as mg of soluble protein g^{-1} of tissue dry weight (DW).

2.4.2. GSH-Px Activity

The antioxidant activity of the enzyme glutathione peroxidase (GSH-Px) was measured using extracts obtained from frozen leaf tissues through the technique described by Paglia and Valentine [35]. The absorbance of the samples was determined at 340 nm using a spectrophotometer. The activity of the GSH-Px enzyme was expressed as U g^{-1} of tissue DW and calculated using the following Equation (2):

$$\boldsymbol{U\,g^{-1}} = \frac{\boldsymbol{\Delta A}}{\boldsymbol{min}} * \boldsymbol{F} \quad (2)$$

where F is a constant used to convert the absorbance per minute ($\Delta A\ min^{-1}$) to enzyme units (U). The F was calculated using the following Equation (3):

$$F = \left(\frac{RV}{SV}\right) * 5/6.22 \quad (3)$$

where RV is the reaction volume (in mL), SV is the volume of the sample (in mL), 5 is the volume (in mL) used to dilute 1 g of tissue during enzymatic extraction, and 6.22 is the NADPH molar extinction coefficient (in mM cm^{-1}).

2.4.3. Total Phenolic Compound

The total phenolic compound content was evaluated using Folin–Ciocalteu reagent by spectrophotometry at 760 nm, as described by Lemoine et al. [36]. This value was expressed as mg gallic acid equivalent g^{-1} of tissue DW. All measurements were performed in triplicate.

2.4.4. Evaluation of Total Antioxidant Capacity

To estimate the antioxidant properties of Se-treated broccoli leaves, the total antioxidant potential of the extracts was determined by a free radical scavenging technique using the ABTS reagent (2,2′-azino-bis-3-ethyl-benzothiazoline-6-sulfonic acid), as described by Kusznierewicz et al. [37]. The antioxidant capacity was expressed as mg ascorbic acid equivalent g^{-1} of tissue DW.

2.4.5. Extraction, Purification, and Quantification of Endogenous Leaves Hormones

The endogenous plant hormones were extracted and purified following the method described by Llugany et al. [38] with some modifications. Briefly, 250 mg of fresh material was ground in an ice-cold mortar with 750 µL extraction solution constituted by MeOH:2-Propanol:HOAc (20:79:1 by vol.). After that, the supernatant was collected after centrifugation at 1000× *g* for 5 min at 4 °C. These steps were repeated two more times and pooled supernatants were lyophilized. Finally, samples were dissolved in 250 µL pure MeOH and filtered with a Spin-X centrifuge tube filter of 0.22 µm cellulose acetate (Costar, Corning Incorporated, New York, NY, USA). Hormone quantification was performed using a standard addition calibration curve spiking control plant samples with the standard solutions of Salicylic acid (SA), (±)-Jasmonic acid (JA), Methyl Jasmonate (MJA), (+)-cis,trans-Abscisic acid (ABA), 3-Indoleacetic Acid (IAA), and Aminocyclopropane-1-carboxylic acid (ACC), ranging from 5 to 250 ppb, and extracted as described above. Deuterated hormones Jasmonic-2,4,4-d3-(acetyl-2,2-d2) acid (JA-d3) and Salicylic acid-d6 (SA-d6) at 30 ppb and 300 ppb, respectively, were used as internal standards in all the samples and standards measurements. All standards were purchased from Sigma-Aldrich® (Barcelona, Spain). Plant hormones were analyzed by LC-ESI-MS/MS system in multiple reaction monitoring mode (MRM) according to Segarra et al. [39]. First, hormones were separated using HPLC Acquity (Waters, Milford, MA, USA) on a Luna Omega 1.6 µm C18 100° 50 × 2.1 mm (Phenomenex®, Waters, USA) at 50 °C at a constant flow rate of 0.8 mL min^{-1} and 10 µL injected volume. The elution gradient was carried out with a binary solvent system consisting of 0.1% formic acid in methanol (solvent A) and 0.1% formic acid in milliQ H_2O (solvent B) with the following proportions (v/v) of solvent A [t (min), %A]: (0, 2), (0.2, 2), (1.6, 100), (2, 100), (2.1, 2), and (3, 2). MS/MS experiments were performed on an ABI 4000 Qtrap mass spectrometer (Perkin-Elmer Sciex, Framingham, MA, USA). All the analyses were performed using the Turbo Ionspray source in negative ion mode except for MJA and ACC.

2.4.6. Total Se and Mineral Elements Determination

Total concentrations of Se, potassium (K), phosphorus (P), boron (B), zinc (Zn), copper (Cu), nickel (Ni), Ca (calcium), Mg (magnesium), S (sulfur), Fe (iron), Mn (manganese), and Mo (molybdenum) were analyzed by Inducted Coupled Plasma Mass Spectrometry (ICP-MS). Prior to analysis, 300 mg of lyophilized tissue (leaves) were acid-digested with 10 mL of a mixture of HNO_3/H_2O_2 (7:3, v/v) in a closed vessel of HP500 PFA at 180 °C and 1.9 atm for 45 min using a microwave digestion system (Mars 5, CEM, Matthews, NC, USA). The digested samples were filtered using 0.22 µm syringe filters and diluted until 3% HNO_3. The samples were analyzed using an Agilent 7900 ICP-MS system (Agilent Technologies, Inc., Lexington, MA, USA). The element S was measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Agilent 5900, USA). The mineral concentration was calculated based on DW and then converted to values based on fresh weight to calculate the recommended daily allowance (RDA, %).

2.5. Preparation of Broccoli Leaf Aqueous Extracts

Nine grams of lyophilized broccoli leaf powder were homogenized in 60 mL of ice-cold distilled water using an immersion blender (SL-SM6038WPN 600 W, Smartlife, Buenos Aires, Argentina) through six pulses of 30 sec each. The homogenate was filtered through a muslin cloth and subjected to centrifugation at 16,090× g for 20 min at 4 °C (Thermo Scientific Sorvall ST16R, Fisher Scientific, Waltham, MA, USA) to remove insoluble debris. This step was repeated to ensure complete clarification. The final volume of the extract was adjusted with distilled water to obtain a stock solution with a concentration of 30% (w/v). The prepared extracts were aliquoted and stored at −20 °C until further use.

2.6. In Vitro Antimicrobial Activity of Aqueous Extracts on the Growth Inhibition of Fusarium solani

To assess the impact of broccoli leaf aqueous extracts on the growth of *Fusarium solani* f. sp. *eumartii* isolate 3122 (EEA-INTA, Balcarce, Argentina), in vitro bioassays were performed following the protocol described by Guevara et al. [40], with some modifications. Briefly, 10 µL of a spore suspension (3×10^5 spores mL^{-1} in distilled water) was mixed with 40 µL of each broccoli leaf extract, selenite (0.485 µg, equivalent to the Se content in 40 µL of selenite-enriched broccoli leaf extract), or distilled water (control), all supplemented with 30% sucrose (w/v). The mixtures, with a final volume of 50 µL, were incubated in darkness at 18 °C for 20 h. Following incubation, samples were observed using bright-field microscopy at 40× magnification with a Nikon Eclipse E200 microscope (Nikon, Tokyo, Japan). Hyphal and spore lengths were measured using ImageJ software v4 [41]. Three independent experiments were conducted, and the hyphal growth index was calculated as the ratio between the average hyphal length and spore length.

2.7. Statistical Analysis

The experimental data were statistically analyzed using InfoStat statistical software version 2017 [42]. The significance of the difference among the mean values was determined by one- or two-way analysis of variance (ANOVA) with subsequent multiple comparisons of means by the least significant difference (LSD) test. A two-way ANOVA was applied to evaluate the effects of foliar Se application and the growth time (Treatment × DAT) on the evolution of morphological parameters (PH and LA). Correct application of ANOVA was checked by residual normal distribution (Shapiro–Wilks test, QQ plots) and homoscedasticity (Levene test, residual plots). Differences at $p < 0.05$ were statistically significant. Data were provided as the meaning from three values for every parameter measured (n = 3) ± standard error.

To obtain an appropriate interpretation of the variable correlation and its relative weight on the results, a multivariate analysis was performed. Thus, principal component analysis (PCA), biplot analysis, and minimum spanning tree (MST) analysis were performed. The quality of the PCA was analyzed by considering the cophenetic correlation coefficient (CCC) value.

3. Results and Discussion

3.1. Evaluation of Changes in Morphological and Physiological Parameters During the Growth Stages of Broccoli Plants

In this study, the impact of foliar Se application on various growth parameters of broccoli plants was evaluated. As detailed in Table 1, Se treatments did not significantly affect PH at 78, 85, or 90 days after transplanting (DAT). These findings suggest that Se application did not change plant height until harvest time (90 DAT), consistent with the results of Muñoz et al. [13]. Our findings also align with those of Ghasemi et al. [15], who reported no significant changes in PH following Se application in broccoli grown under field conditions. Similar observations were made in sesame [43], *Atractylodes macrocephala* [44], *Salvia officinalis* [45], strawberry [46], and tomato [47] after foliar Se applications. These results suggest that foliar Se application at the tested doses does not affect plant height; however, higher doses may inhibit growth, as observed in lettuce [48].

Table 1. Effect of foliar selenium application on the growth parameters (plant height, PH; plant leaf area, LA) of broccoli plants.

Treatment	PH (cm)			LA (cm^2)		
	Days After Transplanting (DAT)					
	78	**85**	**90**	**78**	**85**	**90**
Control	15.0 ± 1.5 fg	19.3 ± 2.8 cdef	24.2 ± 3.1 a	99.5 ± 3.9 abcd	103.0 ± 15.8 abc	107.5 ± 11.7 abc
Selenate 1 mM	13.7 ± 0.9 g	19.5 ± 0.9 bcdef	24.0 ± 1.2 ab	95.9 ± 1.3 bcd	107.17 ± 5.6 abc	107.0 ± 6.2 abcd
Selenate 2 mM	15.5 ± 0.3 efg	18.5 ± 1.5 def	23.3 ± 2.5 abc	102.2 ± 5.1 abcd	121.5 ± 16.8 ab	110.6 ± 22.8 a
Selenite 1 mM	12.2 ± 0.2 g	16.7 ± 0.3 efg	22.3 ± 0.3 abcd	76.9 ± 5.5 d	102.1 ± 10.7 abcd	124.6 ± 16.1 abc
Selenite 2 mM	15.3 ± 0.9 efg	19.7 ± 1.5 abcde	22.2 ± 1.6 abcd	88.9 ± 7.2 cd	103.2 ± 14.6 abcd	91.7 ± 11.8 cd
Treatment × DAT (*p*-value)		0.9542			0.9093	

Note. The results are expressed as the means (n = 3) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance.

Leaf area, a crucial morphological trait contributing significantly to biomass accumulation and photosynthetic efficiency [49], showed no significant differences between treatments at 78 DAT. Similarly, no statistically significant variation in LA was observed at 85 DAT among the different treatments (Table 1). At harvest time (90 DAT), LA showed no marked differences between treatments, consistent with Muñoz et al. [13], who found that foliar Se application did not affect LA in 'Belstar' and 'Legend' cultivars grown under field conditions. This stability in LA across treatments aligns with the natural progression of the plant's developmental stages. At the end of the vegetative stage (68 DAT, when Se application was performed), leaves expand and accumulate photoassimilates, supporting overall plant growth. As the plant transitions to the reproductive phase, leaf expansion

stops, and mature leaves supply photoassimilates to the developing head, a sink for dry matter accumulation [50]. Consequently, these observations suggest that Se does not impact leaf growth once the broccoli plant transitions from the vegetative to the reproductive phase [50–52].

The plant relative elongation rate (PRER), on the other hand, is a straightforward parameter to assess and provides relevant data on variations in plant growth [37]. The foliar application of Se did not modify PRER (Table 2). Previous research has identified PRER as an early indicator of plant stress, often used to detect the onset of adverse conditions [33]. The unchanged PRER values suggest that the foliar Se concentrations used in this study were non-toxic to the plants. The reduction in plant growth and development under abiotic stress, such as flooding, salinity, or toxicity, is often linked to decreased ATP synthesis and reduced photosynthetic rates [53]. Consistently, our previous findings demonstrated that foliar Se application did not negatively affect the photosynthetic rate [13].

Table 2. Changes in the plant relative elongation rate (PRER, cm cm^{-1} d^{-1}) under selenium foliar application treatments.

Treatment	PRER (cm cm^{-1} d^{-1})
Control	0.91 ± 0.09 ab
Selenate 1 mM	0.86 ± 0.04 ab
Selenate 2 mM	0.79 ± 0.15 ab
Selenite 1 mM	0.83 ± 0.01 ab
Selenite 2 mM	0.76 ± 0.02 a

Note. The results are expressed as the means (n = 3) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance.

The chlorophyll index, which indirectly measures leaf chlorophyll content, is a useful indicator for detecting nutrient deficiencies or other stress factors limiting plant growth and productivity [49,54], and showed no significant differences between Se treatments at different growth stages (Table S1). This lack of variation is consistent with Muñoz et al. [13], who concluded that Se-treated plants were not subjected to noticeable stress. However, our findings contrast with those of Palencia et al. [55], who found that Se-treated strawberry plants exhibited older greener leaves compared to controls. The absence of significant changes in CI in our study may be attributed to the fact that plants were harvested at commercial maturity, rather than physiological maturity. In broccoli cultivation, the commercial harvest typically occurs before floret anthesis, when the flower buds are still tightly closed and the head is firm and compact [56]. This stage ensures that the broccoli head is tender and suitable for consumption [57]. However, harvesting at this point means the plant has not yet reached physiological maturity, as it has not completed its full life cycle, including seed production. This distinction could explain the lack of observable effects of Se treatment on leaf greenness, as chlorophyll accumulation in older leaves may be more pronounced when plants are allowed to reach full physiological maturity, as noted by Palencia et al. [55].

3.2. Effect of Foliar Selenium Application on Biomass Distribution, Fresh Weight, Firmness, and Diameter of Broccoli Heads

The foliar application of Se significantly influenced several morphological and physiological parameters in broccoli plants. Both selenate and selenite treatments increased the FW of broccoli heads. Specifically, 1 mM selenate resulted in a 133% increase in FW, while 2 mM selenate and both 1 mM and 2 mM selenite treatments produced a 98% increase compared to the control (Figure S1). These findings contrast with Sindelarova et al. [17],

who reported no significant changes in head FW across various broccoli cultivars ('Heraklion', 'Marathon', 'Parthenon', and 'Naxos') after Se application. However, in the 'Belstar' cultivar analyzed here, the Se treatments positively affected FW, likely by enhancing the water content of the heads, as previously suggested by Muñoz et al. [13]. This indicates that the response to Se treatments is cultivar-dependent and highlights the potential of Se biofortification to improve consumable yield.

In terms of biomass partitioning, DW analysis revealed that Se treatments generally did not alter the dry matter content of leaves or heads, except for a significant increase in stem DW in plants treated with 2 mM selenate and 1 mM selenite (Figure 1). This finding aligns with Muñoz et al. [13], who observed no significant changes in the dry weight of broccoli heads following foliar Se application, despite notable increases in FW. Such effects have been attributed to enhanced water use efficiency (WUE), primarily due to reduced transpiration rates rather than increased photosynthetic activity, as suggested by Muñoz et al. [13].

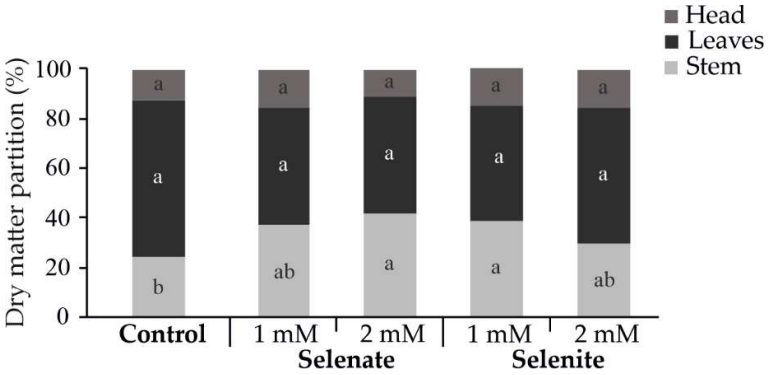

Figure 1. Effect of selenium treatments on the dry matter partitioning of different tissues at harvest. The results are expressed as the means (n = 3) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance.

Head firmness, a critical parameter for commercial quality, was unaffected by Se treatments regardless of dose or salt type (Figure S1). Similarly, HD showed no significant variation across treatments, except for a slight increase under 1 mM selenite (Figure S1). These results corroborate previous reports, which found minimal effects of Se application on head diameter in other broccoli cultivars ('Legend', 'Formoso', and 'Legacy') [13,18,31]. Ghasemi et al. [15] also reported negligible impacts of Se on this parameter, further supporting our findings.

Based on these results, foliar Se application significantly enhances broccoli head FW, primarily by increasing water content, without substantially affecting dry matter content, head firmness, or diameter under the tested conditions. This underscores the commercial advantage of Se biofortification, which improves yield without compromising marketable quality.

3.3. Principal Component Analysis of Selenium Treatments on Growth, Morphological, and Yield Parameters in 'Belstar' Broccoli

The PCA biplot for the broccoli 'Belstar' cultivar (Figure 2) reveals the distribution of growth, morphological, and yield parameters in response to the various Se treatments. Principal components 1 (PC1) and 2 (PC2) together account for 70.9% of the total variation, with PC1 explaining 46.2% and PC2 accounting for 24.7%. The high sum of squares (SC) value of 51.1, a value of 0.986 for CCC, and the distinct separation of the treatments confirm the effectiveness of the multivariate analysis in discriminating between the different Se treatments.

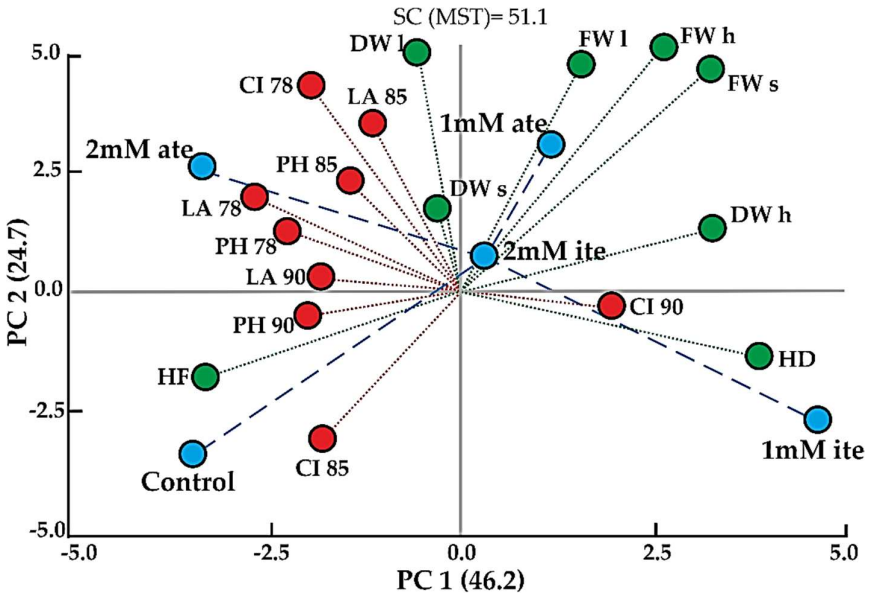

Figure 2. Principal component analysis of broccoli plants grown with foliar application of selenium. Growth parameters measured (red circles): PH, plant height; LA, leaf area; CI, chlorophyll index. DAT: Days after transplant (78, 85, 90). Yield and morphological parameters measured at harvest time (green circles): FW h, head fresh weight; FW s, stem fresh weight; FW l, leaf fresh weight; DW h, head dry weight; DW s, stem dry weight; DW l, leaf dry weight; HF, head firmness; and HD, head diameter. Treatments (blue circles): control, 0 mM selenium; 1 mM ite, 1 mM selenite; 2 mM ite, 2 mM selenite; 1 mM ate, 1 mM selenate; and 2 mM ate, 2 mM selenate. PC 1: Principal Component 1. PC 2: Principal Component 2. SC: sum of squares. MST: minimum spanning tree.

A positive correlation was observed between yield-related parameters such as head fresh weight (FW h), stem fresh weight (FW s), leaf fresh weight (FW l), and head dry weight (DW h), which are located toward the right side along PC1. These parameters showed a strong relationship with higher yields. Additionally, HD is positively associated with these parameters, supporting its role as a marker of higher yield in 'Belstar'.

In contrast, early growth parameters, including LA at 78, 85, and 90 DAT; PH at 78, 85, and 90 DAT; and CI at 85 and 90 DAT, were negatively correlated with yield parameters. These early growth markers are located to the left of PC1 and partially along PC2. This suggests that larger leaf area and plant height, typically associated with early vegetative growth, are inversely related to yield parameters in this cultivar. Head firmness also aligns with these early growth parameters, indicating a negative relationship with the final yield.

The PCA results highlight that the most significant treatment was the application of 1 mM selenite, followed by 2 mM selenite, which led to increased head fresh weight and other yield-related parameters. Conversely, the control treatment and 2 mM selenate were the least effective, as they showed minimal impact on the evaluated parameters, especially head fresh weight. These results align with the treatment rankings derived from the PCA, where 1 mM selenite resulted in the highest head fresh weight, confirming the positive effect of this treatment on the 'Belstar' cultivar.

In conclusion, the PCA clearly demonstrates that Se treatments, particularly 1 mM and 2 mM selenite, positively affect the yield by influencing parameters such as head fresh weight and head diameter. This suggests that the foliar application of Se can enhance broccoli yield, although the specific effects vary depending on the treatment.

Considering the previous report by Muñoz et al. [13], which demonstrated that Se accumulation in broccoli heads is dose-dependent and the significant positive influence of the 2 mM Se dose on the evaluated parameters, we decided to analyze the effect of

this dose on the nutraceutical parameters and plant hormone content in the leaves, as detailed below.

3.4. Selenium Improves Nutraceutical Parameters in Broccoli By-Products

3.4.1. Biochemical Parameters

Broccoli by-products can be used as functional food ingredients in the food industry because of their nutritional qualities [19,20,58,59]. In the present study, the leaves that remained as remnants of plants after the harvest of heads were considered by-products.

Among the parameters of nutritional quality, the level of antioxidants is the most interesting since it plays a critical role in human health because it can prevent oxidative damage to molecules and different cellular components [22]. The total antioxidant capacity significantly increased when the plants were treated with 2 mM selenate compared to that of the control. However, no significant changes were observed when the plants were treated with 2 mM selenite (Table 3).

Table 3. Effect of foliar application of selenium on nutritional quality parameters in broccoli leaves.

	Control	Selenate 2 mM	Selenite 2 mM
Antioxidant capacity (mg ascorbic acid equiv. g^{-1} DW)	2.76 ± 0.11 a	4.38 ± 0.37 b	3.44 ± 0.14 a
GSH-Px activity (U g^{-1} DW)	0.70 ± 0.03 a	1.05 ± 0.13 a	1.20 ± 0.28 a
Phenolic compounds (mg gallic acid equiv. g^{-1} DW)	4.27 ± 0.34 a	6.93 ± 0.84 b	6.53 ± 0.54 b
Soluble proteins content (mg g^{-1} DW)	0.15 ± 0.01 a	0.43 ± 0.02 c	0.24 ± 0.02 b

Note. The results are expressed as the means (n = 3) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance.

In this study, the antioxidant capacity of leaves was also assessed through both enzymatic systems, such as glutathione peroxidase (GSH-Px), and non-enzymatic systems including phenolic compounds. Neither of the Se salt treatments resulted in significant changes in GSH-Px activity compared to the control (Table 3). However, the concentration of phenolic compounds in Se-treated broccoli leaves was significantly higher than in control leaves, regardless of the Se salt applied (Table 3). A strong positive correlation (r = 0.7899) was observed between total antioxidant activity and phenolic compound content, which is consistent with the findings of Hwang and Lim [22], who reported a correlation coefficient of 0.880 for broccoli by-products. Likewise, Domínguez-Perles et al. [19] demonstrated a strong relationship between total phenolic content in broccoli heads and DPPH radical scavenging activity.

The enhancement of the antioxidant status in broccoli leaves following Se treatment observed in this study aligns with the findings of Bouranis et al. [58] and Martirosyan et al. [59], further emphasizing the synergistic relationship between Se and other inherent antioxidants.

Although Se is not considered essential for plants, low concentrations of Se are known to boost antioxidant defense mechanisms in crops [54]. This improvement is directly associated with the plant's ability to mitigate oxidative stress and strengthen defense mechanisms against pathogens. For example, the study on banana plants by Liu et al. [60] highlighted that Se application significantly increased antioxidant capacity, contributing to enhanced resistance against *Fusarium oxysporum*. Furthermore, Liu et al. [60] reported that Se application stimulated the production of resistance-related metabolites and upregulated

defense genes, which could potentially explain the improvements in biochemical properties observed in broccoli.

On the other hand, the increase in phenolic compound levels in the leaves resulting from Se application underscores the nutritional value of the by-products. Among natural antioxidants, phenolic compounds are particularly potent [61], and agricultural products with high polyphenol content have the potential to reduce the risks associated with oxidative stress, such as cancer, inflammation, cardiovascular diseases, and premature aging [61].

Additionally, Se treatments significantly increased the soluble protein content in broccoli leaves (Table 3). The foliar application of 2 mM selenate and 2 mM selenite resulted in a 186% and 60% increase, respectively, compared to the control. Other studies reported that high Se doses in green pea leaves reduced soluble protein content, indicating a potential threshold for Se application beyond which negative nutritional effects may occur [62,63]. In this context, the observed increase in soluble protein content in broccoli leaves facilitated by Se not only enhances their nutritional value but also underscores their potential as a high-quality source of functional ingredients.

3.4.2. Foliar Application of Selenium Increases the Mineral Content of Broccoli By-Products

The ash content in leaves is commonly higher than the ash content in other plant tissues of the same species, so broccoli leaves might be a better source of minerals than the head or the stem [58]. Furthermore, understanding the interactions between Se and other elements in plants is crucial for the development of functional foods with high Se content derived from by-products. Consequently, investigating potential variations in different dietary minerals associated with Se biofortification in plants is of particular importance [62]. The foliar application of Se did not significantly change the mineral concentrations of K, P, B, Zn, Cu, or Ni in broccoli leaves (Table 4), similar to the findings of Golubkina et al. [14] for different Brassicaceae crops.

However, the Se concentration in the leaves increased significantly following the foliar application of both Se species, with a 100-fold increase for 2 mM selenate and a 150-fold increase for 2 mM selenite compared to the control. Similarly, Sindelarova et al. [17] reported that a foliar application of 50 g ha^{-1} selenate (equivalent to 1 mM) significantly increased the total Se content in broccoli leaves by 32.5-fold. Li et al. [64] also found Se concentrations ranging between 1.4 and 3.9 μg g^{-1} DW in untreated broccoli leaves, which were higher than those in the control of this study but lower than those in plants treated with 2 mM selenite and selenate.

On the other hand, variations in Se content can alter absorption pathways or modify the ionic permeability coefficient of the plasma membrane, thereby influencing the accumulation of minerals in plant cells. These changes can manifest as early physiological responses to Se application [65].

Table 4. Mineral concentration (μg g^{-1} DW) in broccoli leaves treated with selenium.

μg g^{-1}	Control	Selenate 2 mM	Selenite 2 mM.
Se	0.27 ± 0.00 a	27.22 ± 0.26 b	40.44 ± 1.37 c
K	17,258 ± 1872.09 a	14,904 ± 393.08 a	18,808 ± 1932.60 a
P	2910 ± 91.53 a	2687 ± 146.30 a	2917 ± 195.65 a
Mg	2811 ± 83.89 ab	2518 ± 60.11 a	3200 ± 143.72 b
Ca	12,809 ± 789.73 a	11,987 ± 519.65 a	19,826 ± 1183.18 b
S	8257 ± 63.98 a	9871 ± 446.20 b	12,971 ± 43.52 c
Fe	27.69 ± 2.27 a	42.66 ± 3.14 b	46.48 ± 2.35 b
Mn	36.61 ± 2.67 a	29.14 ± 3.20 a	52.34 ± 1.62 b
B	36.53 ± 1.79 a	37.47 ± 1.88 a	37.39 ± 3.05 a
Zn	25.66 ± 0.13 a	26.70 ± 0.95 a	25.16 ± 1.87 a
Mo	11.53 ± 0.01 a	9.69 ± 0.69 a	15.30 ± 0.22 b
Cu	2.32 ± 0.36 a	2.41 ± 0.25 a	3.00 ± 0.16 a
Ni	0.30 ± 0.05 a	0.24 ± 0.01 a	0.23 ± 0.01 a

Note. The results are expressed as the means (n = 3) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance.

In this study, foliar concentrations of Ca, Mg, S, Fe, Mn, and Mo were significantly affected by Se treatment compared to the control (Table 4). Selenite treatment significantly increased the levels of all these elements, whereas selenate treatment only led to increased concentrations of S and Fe. These values align with those previously reported for other broccoli cultivars [20].

Saffaryazdi et al. [66] and He et al. [67] demonstrated that selenite biofortification positively affected Ca content in spinach and lettuce, respectively, although the magnitude of the increase varied among species. Similarly, Rios et al. [68] reported significant differences in Ca content in hydroponically grown lettuce biofortified with either selenite or selenate. However, both Se species reduced Ca levels compared to untreated controls. The increase in Ca observed in this study may be attributed to the ions' role in regulating cell membrane potential and turgor [69]. Magnesium also plays a protective role in maintaining the integrity of plant tissues [70] as part of a Se tolerance mechanism [71]. The observed increase in Mg levels in broccoli leaves could suggest this possible involvement. In contrast, Boldrin et al. [72] found that soil-applied selenate and selenite significantly increased Mg content in rice, whereas foliar applications had no significant effect. This discrepancy may be attributed to shorter exposure times in foliar applications compared to soil treatments [63]. Conversely, Rios et al. [68] observed decreased Mg content in lettuce under hydroponic conditions with both Se species. These findings emphasize the importance of application methods, as Se biofortification affects not only Se accumulation but also the levels of other essential minerals [73,74].

Sulfur is a particularly relevant nutrient in the context of Se biofortification, as selenate is absorbed through sulfate transporters [75]. Freeman et al. [76] observed that in Se-treated *Stanleya pinnata*, the molecular mechanisms controlling Se accumulation involved a greater expression of genes related to S assimilation, which could explain the elevated S levels observed in this study. Similarly, Hawrylak-Nowak [75] reported a significant increase in S content in the shoots of hydroponically grown lettuce treated with selenate, consistent with the results observed for broccoli under foliar Se application (Table 4).

Selenium, along with trace elements such as Mn, Zn, Fe, and Cu, serves as a cofactor for human endogenous antioxidant enzymes [77]. The impact of Se biofortification on these trace minerals suggests that Se-enriched foods could influence antioxidant capacities. Boldrin et al. [72] observed that soil-applied selenate significantly increased Mn and Zn content in rice grains. While Mn levels often rise or remain stable following Se biofortification, Zn levels typically show no significant changes. In this study, Mn content in

broccoli leaves increased significantly after foliar selenite application, whereas Zn levels remained unaffected (Table 4), highlighting the importance of the application method. Furthermore, in Se-biofortified *B. napus*, the expression of the ZRT/IRT family member (IRT1) was upregulated under selenite treatments, facilitating Mn translocation [78]. This mechanism could explain the higher Mn content observed in broccoli leaves.

Another metabolic pathway influenced by Se is the variation in cellular redox balance [73], which involves antioxidant synthesis associated with the reduction in reactive oxygen species (ROS) levels [79]. These changes are likely due to alterations in the concentrations of microelements acting as cofactors for various enzymes, including superoxide dismutase (Fe, Mn, Cu, and Zn), peroxidase (Fe), and catalase (Fe) [79]. Depending on the Se concentration, either a pro-oxidant or antioxidant response may be triggered, modifying gene expression and altering transcript abundance and the post-transcriptional regulation of various proteins, including transport proteins [80]. These mechanisms could partially explain the variability observed in the concentrations of different elements in plants influenced by Se application. For instance, high Se concentrations have been reported to increase Fe absorption, whereas low Se concentrations reduce it in *Pteris vittata* [81]. Additionally, soil-applied Se has shown no significant impact on Fe content in rice, whereas foliar selenate application significantly increased Fe levels [68]. Conversely, He et al. [67] reported that soil-applied selenite reduced Fe content in lettuce, while Rios et al. [68] observed increased Fe content in hydroponically grown lettuce treated with both Se species. These findings suggest a general trend of higher Fe content in hydroponic systems and lower or unchanged levels under soil applications [63].

Regarding Cu, its content typically decreases or remains stable with Se biofortification, as observed in this study. However, Rios et al. [68] reported increased Cu levels in lettuce grown hydroponically with selenite. In general, Se biofortification can positively or negatively affect the uptake of various minerals essential to human health [63]. Identifying dietary minerals negatively impacted by Se biofortification is critical to assess potential reductions in their dietary intake. Furthermore, given the high variability in application techniques across studies measuring mineral content, additional research is needed to develop optimized biofortification strategies for individual plant species.

Overall, these findings emphasize the complex interactions between Se biofortification and mineral uptake. Selenium can modify transport and assimilation pathways for various elements, influencing their concentrations in plant tissues. Understanding these mechanisms is critical to optimizing Se biofortification strategies for specific plant species, ensuring not only improved Se content but also balanced nutritional profiles.

Finally, the recommended daily allowance (RDA, %) was calculated for selenite-treated leaves based on fresh tissue (100 g) according to Liu et al. [20]. The RDA of Fe (4.9%), Zn (4.42%), Mn (44%), Ca (31.86%), S (11.8%), and Mg (14.7%) was significantly greater than that of the controls. In this way, it was shown that the foliar application of Se not only increases the content of this essential element but also improves the nutritional quality of broccoli by-products through the accumulation of other beneficial mineral elements for human health.

3.5. Differential Effects of Selenate and Selenite on Phytohormone Regulation in Broccoli Leaves: Balancing Growth and Defense Responses

The application of Se treatments significantly influenced the concentrations of phytohormones in broccoli leaves, as outlined in Table 5. Specifically, MJA levels increased substantially under both selenate and selenite treatments compared to the control. This increase suggests that Se may enhance plant defense mechanisms, as MJA plays a pivotal role in stress and defense signaling pathways [81]. In contrast, JA levels were reduced under selenate treatment, while selenite treatment resulted in intermediate levels. This

reduction in JA may reflect the complex regulatory role of Se in modulating jasmonate pathways, potentially balancing plant growth and defense responses, as noted by Skrypnik et al. [45], who discussed Se's impact on the modulation of growth–defense trade-offs in plants.

Table 5. Phytohormone concentration (ng g^{-1} DW) in broccoli leaves treated with selenium for methyl jasmonate (MJA), jasmonic acid (JA), salicylic acid (SA), 3-indoleacetic acid (IAA), abscisic acid (ABA), and aminocyclopropane-1-carboxylic acid (ACC).

ng g^{-1}	Control	Selenate 2 mM	Selenite 2 mM
[MJA]	15.05 ± 0.92 a	19.86 ± 3.03 ab	28.99 ± 2.43 b
[JA]	61.52 ± 5.74 b	40.54 ± 2.89 a	54.04 ± 6.32 ab
[SA]	3216.33 ± 204.84 a	3306.33 ± 112.72 a	9793.00 ± 633.33 b
[IAA]	12.27 ± 2.54 a	27.38 ± 3.47 b	10.87 ± 0.91 a
[ABA]	51.08 ± 3.54 b	97.44 ± 6.38 c	28.84 ± 6.51 a
[ACC]	5.85 ± 0.29 b	6.75 ± 0.68 b	3.55 ± 0.60 a

Note. The results are expressed as the means (n = 3) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance.

Salicylic acid concentrations increased significantly under selenite treatment compared to both the control and selenate treatments. Elevated SA levels are often associated with enhanced systemic acquired resistance and improved pathogen defense [82]. The marked increase in SA under selenite treatment suggests that this species of Se may be particularly effective in activating SA-mediated defense pathways [83].

Indole-3-acetic acid levels were significantly higher under selenate treatment compared to both the control and selenite treatments. As IAA is a critical hormone involved in cell elongation and division, its elevation suggests that selenate promotes vegetative growth and development under non-toxic Se concentrations [45]. Additionally, ABA concentrations were significantly elevated under selenate treatment, highlighting a key role for ABA in mediating stress responses such as drought and salinity tolerance. The increase in ABA under selenate treatment suggests that Se may enhance the plant's capacity to mitigate abiotic stresses, aligning with the findings of Chao et al. [82], who linked Se application with enhanced stress resilience in plants.

Conversely, ACC, a precursor of ethylene, exhibited lower levels under selenite treatment compared to both the control and selenate treatments. Ethylene is typically involved in stress responses and senescence, and the suppression of ACC under selenite may alleviate stress-induced growth inhibition [45]. This suggests that selenite's regulatory effect on ethylene biosynthesis could enhance growth by reducing the impact of stress-related signals [84], promoting plant growth.

These results align with the findings of Chao et al. [82], who reported that Se could enhance plant defense systems through hormonal regulation, particularly by increasing MJA and SA. The observed increase in SA under selenite treatment supports this notion, as SA is known to activate systemic acquired resistance pathways that improve pathogen defense. Similarly, the rise in IAA and ABA under selenate treatment corroborates the work of Skrypnik et al. [45], who discussed Se's role in regulating secondary metabolism and stress adaptation. These findings further highlight the differential effects of selenate and selenite on hormone regulation, underscoring the importance of Se speciation in determining its physiological impact on plant growth and defense mechanisms.

The distinct effects of selenate and selenite on hormonal regulation demonstrate the potential for tailoring Se supplementation strategies in agriculture. While selenate enhances growth-related hormones (IAA and ABA), selenite primarily affects defense-

related hormones (MJA and SA), emphasizing the need for an optimal balance between growth promotion and defense activation to improve plant resilience and productivity.

3.6. Antifungal Potential of Selenium-Biofortified Broccoli Leaf Extracts Against Fusarium solani

Building on the previously conducted biochemical (Table 3) and hormonal analysis of broccoli leaves (Table 5), which highlights their association with plant defense mechanisms, this study investigated the antifungal potential of aqueous extracts from untreated and 2 mM selenite-treated broccoli leaves (Figure 3). The results demonstrated that both treatments, the leaf extract (LE) and the selenite-treated leaf extract (Selenite-LE), significantly inhibited the hyphal growth of *F. solani* compared to the control and the selenite-only treatment. Specifically, LE, corresponding to an aqueous extract of untreated broccoli leaves, achieved approximately 40% inhibition of fungal growth relative to the control. In contrast, Selenite-LE, derived from broccoli leaves treated with 2 mM selenite, exhibited a substantially higher inhibitory effect, with fungal growth inhibition of approximately 67.5% compared to the control. However, the selenite-only treatment exhibited a weak inhibitory effect, which was not significantly different from the control. This indicates that while Se alone may contribute to fungal growth inhibition [85–87], its effect is less pronounced than that observed with the biofortified extracts.

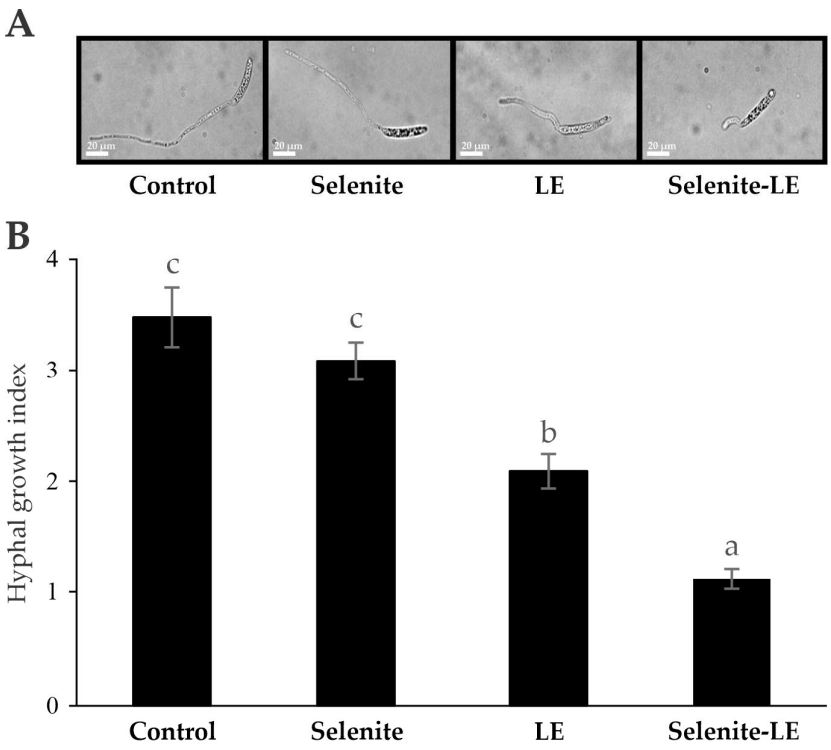

Figure 3. Effect of broccoli leaf aqueous extracts on *F. solani* hyphal growth. (**A**) Spores of *F. solani* were incubated in the presence of water (control), selenite (9.7 ŋg μL^{-1}), aqueous extracts of untreated broccoli leaves (LE), or selenite-treated broccoli leaves (Selenite-LE). After incubation, the morphological changes were analyzed by bright-field microscopy (40×). (**B**) The hyphal growth index was calculated as ratio between the average lengths of hyphal and spore. The results are expressed as the means (n = 25) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance.

A comparison with previous findings by Hanson et al. [88] revealed that *B. juncea* seedlings treated with Se and subsequently infected with a *Fusarium* spore suspension experienced less biomass compared to untreated seedlings, despite both groups exhibiting infection symptoms. Furthermore, higher Se concentrations inhibited *Fusarium* growth,

suggesting that Se may directly suppress fungal development. These findings are consistent with the inhibitory effects observed in the present study, supporting the hypothesis that Se enhances the antifungal properties of aqueous broccoli leaf extracts.

Several studies have demonstrated Se's potential to mitigate the harmful effects of pathogens. For example, Se has been shown to effectively reduce fungal diseases caused by *Fusarium* spp. and *Sclerotinia sclerotiorum* in Brassicaceae species (*B. juncea* and *B. napus*), Solanaceae species (*Solanum lycopersicum*), and Helianthus species (*Helianthus annuus*) [88–91]. In a related study, Somalraju et al. [92] demonstrated that foliar Se application reduced the severity and incidence of late blight in potato plants. Also, Se has been shown to inhibit the growth of several fungal pathogens, including *F. oxysporum*, *F. graminearum*, *Botrytis cinerea*, *Aspergillus flavus*, and *Penicillium expansum* [87,91,93,94].

This protective effect was attributed to Se's dual role as a pathogen growth inhibitor and an elicitor of induced plant defense mechanisms. This evidence suggests that Se's antifungal efficacy may extend beyond *F. solani* to other plant–pathogen interactions.

Furthermore, selenium nanoparticles (Se-NPs) have been reported to exhibit significant antimicrobial activity, as demonstrated by Nowruzi et al. [95]. This indicates that the antimicrobial efficacy of Se can vary depending on its chemical species and application method, thereby opening avenues for further optimization.

Taken together, these findings highlight the potential of Se, particularly via foliar application, to enhance plant defense responses and inhibit fungal pathogen growth, supporting its use as a sustainable strategy in plant disease management [88,96].

4. Conclusions

The present study demonstrates the significant potential of Se biofortification in broccoli, particularly with 2 mM selenite, to enhance both the nutritional and functional properties of broccoli by-products. Se biofortification increased Se content, antioxidant capacity, phenolic compounds, soluble proteins, and essential minerals such as calcium, magnesium, sulfur, and iron. Additionally, Se-enriched broccoli leaves exhibited antifungal activity against *F. solani*, highlighting their potential as natural biopesticides. These findings emphasize the dual benefits of Se biofortification, improving human health through enhanced nutritional quality while simultaneously supporting sustainable agricultural practices by valorizing crop residues. This strategy not only addresses global Se deficiencies but also promotes environmental sustainability. The results advocate for the adoption of Se biofortification as an agronomic and industrial approach with potential applications in both health and sustainability. Future research should further explore the integration of Se-enriched by-products into functional foods and agricultural practices to maximize these benefits.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy15020389/s1, Figure S1: Effect of selenium treatments on (**A**) head fresh weight, (**B**) head firmness, and (**C**) head diameter at harvest. The results are expressed as the means ($n = 3$) ± standard errors. The means not sharing any letter are significantly different according to the LSD test at the $p < 0.05$ level of significance; Table S1: Effects of foliar application of selenium on leaf chlorophyll index (SPAD) of broccoli.

Author Contributions: Conceptualization, G.C., L.D.D. and F.F.M.; Formal analysis, M.S.B., G.C., B.S.T., M.M.S., M.S., V.R.T., L.D.D. and F.F.M.; Investigation, M.S.B., G.C., B.S.T., M.M.S., M.S., C.A.B., V.R.T., M.L., M.-J.S.-M. and F.F.M.; Resources, C.A.B. and A.A.P.; Writing—original draft, M.S.B., B.S.T., M.M.S., M.S., C.A.B., V.R.T., A.A.P. and L.D.D.; Writing—review and editing, G.C., M.L., M.-J.S.-M., M.G.G. and F.F.M.; Supervision, C.A.B., M.L., M.-J.S.-M. and M.G.G.; Project administration, F.F.M.; Funding acquisition, G.C., L.D.D. and F.F.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación, grant number PICT-2015-0142; Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, grant number PIP 2021-2023 11220200100488CO; and Universidad Nacional del Litoral, grant number CAI+D2020 50520190100151LI.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors upon request.

Acknowledgments: The authors thank the Se4All project funded by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No. 101007630. The assistance of Carla Borghese, Gabriela Ochoa, and Mauro Alisio is gratefully appreciated.

Conflicts of Interest: The authors declare that the present work was conducted in the absence of any commercial or financial relationship that could be considered a potential conflict of interest. All authors read and approved the final manuscript.

References

1. Hariharan, S.; Dharmaraj, S. Selenium and selenoproteins: It's role in regulation of inflammation. *Inflammopharmacology* **2020**, *28*, 667–695. [CrossRef] [PubMed]
2. Avery, J.C.; Hoffmann, P.R. Selenium, Selenoproteins, and Immunity. *Nutrients* **2018**, *10*, 1203. [CrossRef] [PubMed]
3. Tinggi, U. Selenium: Its role as antioxidant in human health. *Environ. Health Prev. Med.* **2008**, *13*, 102–108. [CrossRef] [PubMed]
4. Bjørklund, G.; Shanaida, M.; Lysiuk, R.; Antonyak, H.; Klishch, I.; Shanaida, V.; Peana, M. Selenium: An Antioxidant with a Critical Role in Anti-Aging. *Molecules* **2022**, *27*, 6613. [CrossRef]
5. Galić, L.; Vinković, T.; Ravnjak, B.; Lončarić, Z. Agronomic Biofortification of Significant Cereal Crops with Selenium—A Review. *Agronomy* **2021**, *11*, 1015. [CrossRef]
6. Wan, H.-S.; Zhang, W.-C.; Wu, W.; Liu, H.-B. The Controlling Factors of Soil Selenium Content in a Selenium-Deficient Area in Southwest China. *Agronomy* **2023**, *13*, 1031. [CrossRef]
7. Bañuelos, G.S.; Lin, Z.-Q.; Broadley, M. Selenium Biofortification. In *Selenium in Plants: Molecular, Physiological, Ecological and Evolutionary Aspects*; Pilon-Smits, E.A.H., Winkel, L.H.E., Lin, Z.-Q., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 231–255.
8. Poblaciones, M.J.; Broadley, M.R. Foliar selenium biofortification of broccolini: Effects on plant growth and mineral accumulation. *J. Hortic. Sci. Biotechnol.* **2022**, *97*, 730–738. [CrossRef]
9. Subirana, M.A.; Boada, R.; Xiao, T.; Llugany, M.; Valiente, M. Direct and indirect selenium speciation in biofortified wheat: A tale of two techniques. *Physiol. Plant.* **2023**, *175*, e13843. [CrossRef]
10. Bañuelos, G.S.; Arroyo, I.; Pickering, I.J.; Yang, S.I.; Freeman, J.L. Selenium biofortification of broccoli and carrots grown in soil amended with Se-enriched hyperaccumulator *Stanleya pinnata*. *Food Chem.* **2015**, *166*, 603–608. [CrossRef]
11. Bañuelos, G.S.; Arroyo, I.S.; Dangi, S.R.; Zambrano, M.C. Continued Selenium Biofortification of Carrots and Broccoli Grown in Soils Once Amended with Se-Enriched *S. pinnata*. *Front. Plant Sci.* **2016**, *7*, 1251. [CrossRef]
12. D'Amato, R.; Regni, L.; Falcinelli, B.; Mattioli, S.; Benincasa, P.; Dal Bosco, A.; Pacheco, P.; Proietti, P.; Troni, E.; Santi, C.; et al. Current Knowledge on Selenium Biofortification to Improve the Nutraceutical Profile of Food: A Comprehensive Review. *J. Agric. Food Chem.* **2020**, *68*, 4075–4097. [CrossRef] [PubMed]
13. Muñoz, F.F.; Stoffel, M.M.; Céccoli, G.; Trod, B.S.; Daurelio, L.D.; Bouzo, C.A.; Guevara, M.G. Improving the foliar biofortification of broccoli with selenium without commercial quality losses. *Crop Sci.* **2021**, *61*, 4218–4228. [CrossRef]
14. Golubkina, N.; Antoshkina, M.; Bondareva, L.; Sekara, A.; Campagna, E.; Caruso, G. Effect of Foliar Application of Sodium Selenate on Mineral Relationships in Brassicaceae Crops. *Horticulturae* **2023**, *9*, 535. [CrossRef]
15. Ghasemi, Y.; Ghasemi, K.; Pirdashti, H.; Asgharzadeh, R. Effect of selenium enrichment on the growth, photosynthesis and mineral nutrition of broccoli. *Not. Sci. Biol.* **2016**, *8*, 109–203. [CrossRef]
16. Hsu, F.C.; Wirtz, M.; Heppel, S.C.; Bogs, J.; Krämer, U.; Khan, M.S.; Bub, A.; Hell, R.; Rausch, T. Generation of Se-fortified broccoli as functional food: Impact of Se fertilization on S metabolism. *Plant Cell Environ.* **2011**, *34*, 192–207. [CrossRef]
17. Sindelarova, K.; Szakova, J.; Tremlova, J.; Mestek, O.; Praus, L.; Kana, A.; Najmanova, J.; Tlustos, P. The response of broccoli (*Brassica oleracea* convar. *italica*) varieties on foliar application of selenium: Uptake, translocation, and speciation. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2015**, *32*, 2027–2038. [CrossRef]
18. Lozano, L.; Tálamo, A.; Artinián, A.L.; Fernández, J.; Arroyo, C. Evaluación de dos híbridos de brócoli (*Brassica oleracea* var. italica). *Horticultura* **2017**, *36*, 37–48.

19. Dominguez-Perles, R.; Martinez-Ballesta, M.C.; Carvajal, M.; Garcia-Viguera, C.; Moreno, D.A. Broccoli derived by-products a promising source of bioactive ingredients. *J. Food Sci.* **2010**, *75*, C383–C392. [CrossRef]
20. Liu, M.; Zhang, L.; Ser, S.L.; Cumming, J.R.; Ku, K.M. Comparative Phytonutrient Analysis of Broccoli By-Products: The Potentials for Broccoli By-Product Utilization. *Molecules* **2018**, *23*, 900. [CrossRef]
21. Artés-Hernández, F.; Martínez-Zamora, L.; Cano-Lamadrid, M.; Hashemi, S.; Castillejo, N. Genus Brassica By-Products Revalorization with Green Technologies to Fortify Innovative Foods: A Scoping Review. *Foods* **2023**, *12*, 561. [CrossRef]
22. Hwang, J.H.; Lim, S.B. Antioxidant and anticancer activities of broccoli by-products from different cultivars and maturity stages at harvest. *Prev. Nutr. Food Sci.* **2015**, *20*, 8–14. [CrossRef] [PubMed]
23. Francioso, O. Current and future perspectives for biomass waste management and utilization. *Sci. Rep.* **2024**, *14*, 9635. [CrossRef] [PubMed]
24. Gudiño, I.; Martín, A.; Casquete, R.; Prieto, M.H.; Ayuso, M.C.; Córdoba, M.G. Evaluation of broccoli (*Brassica oleracea* var. italica) crop by-products as sources of bioactive compounds. *Sci. Hortic.* **2022**, *304*, 111284. [CrossRef]
25. Tripathi, N.; Hills, C.D.; Singh, R.S.; Atkinson, C.J. Biomass waste utilisation in low-carbon products: Harnessing a major potential resource. *npj Clim. Atmos. Sci.* **2019**, *2*, 35. [CrossRef]
26. Sadh, P.K.; Chawla, P.; Kumar, S.; Das, A.; Kumar, R.; Bains, A.; Sridhar, K.; Duhan, J.S.; Sharma, M. Recovery of agricultural waste biomass: A path for circular bioeconomy. *Sci. Total Environ.* **2023**, *870*, 161904. [CrossRef]
27. Zhou, C.; Wang, Y. Recent progress in the conversion of biomass wastes into functional materials for value-added applications. *Sci. Technol. Adv. Mater.* **2020**, *21*, 787–804. [CrossRef]
28. Shinali, T.S.; Zhang, Y.; Altaf, M.; Nsabiyeze, A.; Han, Z.; Shi, S.; Shang, N. The Valorization of Wastes and Byproducts from Cruciferous Vegetables: A Review on the Potential Utilization of Cabbage, Cauliflower, and Broccoli Byproducts. *Foods* **2024**, *13*, 1163. [CrossRef]
29. Damas-Job, M.C.; Soriano-Melgar, L.A.A.; Rodríguez-Herrera, R.; Peralta-Rodríguez, R.D.; Rivera-Cabrera, F.; Martínez-Vazquez, D.G. Effect of broccoli fresh residues-based extracts on the postharvest quality of cherry tomato (*Solanum lycopersicum* L.) fruits. *Sci. Hortic.* **2023**, *317*, 112076. [CrossRef]
30. Eugui, D.; Velasco, P.; Abril-Urías, P.; Escobar, C.; Gómez-Torres, Ó.; Caballero, S.; Poveda, J. Glucosinolate-extracts from residues of conventional and organic cultivated broccoli leaves (*Brassica oleracea* var. *italica*) as potential industrially-scalable efficient biopesticides against fungi, oomycetes and plant parasitic nematodes. *Ind. Crops Prod.* **2023**, *200*, 116841. [CrossRef]
31. Trod, B.S.; Buttarelli, M.S.; Stoffel, M.M.; Céccoli, G.; Olivella, L.; Barengo, P.B.; Llugany, M.; Guevara, M.G.; Muñoz, F.F.; Daurelio, L.D. Postharvest commercial quality improvement of broccoli (*Brassica oleracea* L.) after foliar biofortification with selenium. *Crop Sci.* **2023**, *63*, 784–800. [CrossRef]
32. Meier, U. (Ed.) Growth stages of mono-and dicotyledonous plants. In *BBCH Monograph*, 4th ed.; Federal Biological Research Centre for Agriculture and Forestry: Quedlinburg, Germany; Julius Kuhn-Institut: Quedlinburg, Germany, 2018; p. 204.
33. Céccoli, G.; Seen, M.; Bustos, D.; Ortega, L.I.; Cordoba, A.; Vegetti, A.; Taleisnik, E. Genetic variability for responses to short- and long-term salt stress in vegetative sunflower plants. *J. Plant Nutr. Soil Sci.* **2012**, *175*, 882–890. [CrossRef]
34. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]
35. Paglia, D.E.; Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158–169. Available online: https://pubmed.ncbi.nlm.nih.gov/6066618/ (accessed on 1 January 2025). [PubMed]
36. Lemoine, M.; Chaves, A.R.; Martínez, G. Influence of combined hot air and UV-C treatment on the antioxidant system of minimally processed broccoli (*Brassica oleracea* L. var. italica). *LWT—Food Sci. Technol.* **2010**, *43*, 1313–1319. [CrossRef]
37. Kusznierewicz, B.; Bartoszek, A.; Wolska, L.; Drzewiecki, J.; Gorinstein, S.; Namieśnik, J. Partial characterization of white cabbages (*Brassica oleracea* var. *capitata* f. *alba*) from different regions by glucosinolates, bioactive compounds, total antioxidant activities and proteins. *LWT—Food Sci. Technol.* **2008**, *41*, 1–9. [CrossRef]
38. Llugany, M.; Martin, S.R.; Barceló, J.; Poschenrieder, C. Endogenous jasmonic and salicylic acids levels in the Cd-hyperaccumulator Noccaea (Thlaspi) praecox exposed to fungal infection and/or mechanical stress. *Plant Cell Rep.* **2013**, *32*, 1243–1249. [CrossRef]
39. Segarra, G.; Jáuregui, O.; Casanova, E.; Trillas, I. Simultaneous quantitative LC–ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of *Cucumis sativus* under biotic stress. *Phytochemistry* **2006**, *67*, 395–401. [CrossRef]
40. Guevara, M.G.; Oliva, C.R.; Huarte, M.; Daleo, G.R. An aspartic protease with antimicrobial activity is induced after infection and wounding in intercellular fluids of potato tubers. *Eur. J. Plant Pathol.* **2002**, *108*, 131–137. [CrossRef]
41. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [CrossRef]
42. Di Rienzo, J.; Casanoves, F.; Balzarini, M.; Gonzalez, L.; Tablada, M.; Robledo, C. *InfoStat. Statistical Software*; Grupo Infostat FCA UNC: Córdoba, Argentina, 2017.

43. Thuc, L.V.; Sakagami, J.I.; Hung, L.T.; Huu, T.N.; Khuong, N.Q.; Vi, L.L.V. Foliar selenium application for improving drought tolerance of sesame (*Sesamum indicum* L.). *Open Agric.* **2021**, *6*, 93–101. [CrossRef]
44. Zhou, W.; Duan, Y.; Zhang, Y.; Wang, H.; Huang, D.; Zhang, M. Effects of foliar selenium application on growth and rhizospheric soil micro-ecological environment of *Atractylodes macrocephala* Koidz. *S. Afr. J. Bot.* **2021**, *137*, 98–109. [CrossRef] [PubMed]
45. Skrypnik, L.; Feduraev, P.; Golubkina, N.; Maslennikov, P.; Antipina, M.; Katserov, D.; Murariu, O.C.; Tallarita, A.V.; Caruso, G. Foliar spraying of selenium in inorganic and organic forms stimulates plant growth and secondary metabolism of sage (*Salvia officinalis* L.) through alterations in photosynthesis and primary metabolism. *Sci. Hortic.* **2024**, *338*, 113633. [CrossRef]
46. Lin, Y.; Cao, S.; Wang, X.; Liu, Y.; Sun, Z.; Zhang, Y.; Li, M.; Wang, Y.; He, W.; Zhang, Y.; et al. Foliar application of sodium selenite affects the growth, antioxidant system, and fruit quality of strawberry. *Front. Plant Sci.* **2024**, *15*, 1449157. [CrossRef] [PubMed]
47. Hu, W.; Su, Y.; Yang, R.; Xie, Z.; Gong, H. Effect of Foliar Application of Silicon and Selenium on the Growth, Yield and Fruit Quality of Tomato in the Field. *Horticulturae* **2023**, *9*, 1126. [CrossRef]
48. Cheng, H.; Shi, X.; Li, L. The Effect of Exogenous Selenium Supplementation on the Nutritional Value and Shelf Life of Lettuce. *Agronomy* **2024**, *14*, 1380. [CrossRef]
49. Liu, C.; Liu, Y.; Lu, Y.; Liao, Y.; Nie, J.; Yuan, X.; Chen, F. Use of a leaf chlorophyll content index to improve the prediction of above-ground biomass and productivity. *PeerJ* **2019**, *2019*, e6240. [CrossRef]
50. Francescangeli, N.; Sangiacomo, M.; Marti, H. Effects of plant density in broccoli on yield and radiation use efficiency. *Sci. Hortic.* **2006**, *110*, 135–143. [CrossRef]
51. Huang, J.; Sedano, F.; Huang, Y.; Ma, H.; Li, X.; Liang, S.; Tian, L.; Zhang, X.; Fan, J.; Wu, W. Assimilating a synthetic Kalman filter leaf area index series into the WOFOST model to improve regional winter wheat yield estimation. *Agric. For. Meteorol.* **2016**, *216*, 188–202. [CrossRef]
52. Lindemann-Zutz, K.; Fricke, A.; Stützel, H. Prediction of time to harvest and its variability of broccoli (*Brassica oleracea* var. italica) part II. Growth model description, parameterisation and field evaluation. *Sci. Hortic.* **2016**, *200*, 151–160. [CrossRef]
53. Casierra-Posada, F.; Peña-Olmos, J.E. Prolonged waterlogging reduces growth and yield in Broccoli Plants (*Brassica oleracea* var. *italica*). *Gesunde Pflanz.* **2022**, *74*, 249–257. [CrossRef]
54. El-Mogy, M.M.; Mahmoud, A.W.M.; El-Sawy, M.B.I.; Parmar, A. Pre-Harvest Foliar Application of Mineral Nutrients to Retard Chlorophyll Degradation and Preserve Bio-Active Compounds in Broccoli. *Agronomy* **2019**, *9*, 711. [CrossRef]
55. Palencia, P.; Martinez, F.; Burducea, M.; Oliveira, J.A.; Giralde, I. Efectos del enriquecimiento con Selenio en spad, calidad de la fruta y parámetros de crecimiento de plantas de fresa en un sistema de cultivo sin suelo. *Rev. Bras. Frutic.* **2016**, *38*, 202–212. [CrossRef]
56. Conversa, G.; Lazzizera, C.; Bonasia, A.; Elia, A. Harvest Season and Genotype Affect Head Quality and Shelf-Life of Ready-to-Use Broccoli. *Agronomy* **2020**, *10*, 527. [CrossRef]
57. Dixon, G.; Wells, R. *Vegetable Brassicas and Related Crucifers*, 2nd ed.; CABI Digital Library: Wallingford, UK, 2024; p. 544. [CrossRef]
58. Bouranis, D.L.; Stylianidis, G.P.; Manta, V.; Karousis, E.N.; Tzanaki, A.; Dimitriadi, D.; Bouzas, E.A.; Siyiannis, V.F.; Constantinou-Kokotou, V.; Chorianopoulou, S.N.; et al. Floret Biofortification of Broccoli Using Amino Acids Coupled with Selenium under Different Surfactants: A Case Study of Cultivating Functional Foods. *Plants* **2023**, *12*, 1272. [CrossRef]
59. Martirosyan, V.V.; Kostyuchenko, M.N.; Kryachko, T.I.; Malkina, V.D.; Zhirkova, E.V.; Golubkina, N.A. The Beneficial Effect of Selenium-Enriched Broccoli on the Quality Characteristics of Bread. *Processes* **2023**, *11*, 3037. [CrossRef]
60. Liu, L.; Wang, C.; Yin, K.; Ni, M.; Ding, Y.; Li, C.; Zheng, S.-J. The Dual Effect of Selenium Application in Reducing Fusarium Wilt Disease Incidence in Banana and Producing Se-Enriched Fruits. *Plants* **2024**, *13*, 3435. [CrossRef]
61. Tsao, R. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients* **2010**, *2*, 1231–1246. [CrossRef]
62. Garousi, F.; Domokos-Szabolcsy, E.; Jánószky, M.; Balláné Kovács, A.; Veres, S.; Soós, A.; Kovács, B. Selenoamino acid-enriched Green Pea as a value-added plant protein source for humans and livestock. *Plant Foods Hum. Nutr.* **2017**, *72*, 168–175. [CrossRef]
63. Newman, R.; Waterland, N.; Moon, Y.; Tou, J.C. Selenium biofortification of agricultural crops and effects on plant nutrients and bioactive compounds important for human health and disease prevention—A Review. *Plant Foods Hum. Nutr.* **2019**, *74*, 449–460. [CrossRef]
64. Li, H.; Xia, Y.; Liu, H.Y.; Guo, H.; He, X.Q.; Liu, Y.; Wu, D.T.; Mai, Y.H.; Li, H.B.; Zou, L.; et al. Nutritional values, beneficial effects, and food applications of broccoli (*Brassica oleracea* var. *italica* Plenck). *Trends Food Sci. Technol.* **2022**, *119*, 288–308. [CrossRef]
65. Pazurkiewicz-Kocot, K.; Kita, A.; Pietruszka, M. Effect of Selenium on Magnesium, Iron, Manganese, Copper, and Zinc Accumulation in Corn Treated by Indole-3-acetic Acid. *Commun. Soil Sci. Plant Anal.* **2008**, *39*, 2303–2318. [CrossRef]
66. Saffaryazdi, A.; Lahouti, M.; Ganjeali, A.; Bayat, H. Impact of selenium supplementation on growth and selenium accumulation on spinach (*Spinacia oleracea* L.) plants. *Not. Sci. Biol.* **2012**, *4*, 95–100. [CrossRef]
67. He, P.P.; Lv, X.Z.; Wang, G.Y. Effects of Se and Zn supplementation on the antagonism against Pb and Cd in vegetables. *Environ. Int.* **2004**, *30*, 167–172. [CrossRef] [PubMed]

68. Rios, J.J.; Blasco, B.; Leyva, R.; Sanchez-Rodriguez, E.; Rubio-Wilhelmi, M.M.; Romero, L.; Ruiz, J.M. Nutritional balance changes in lettuce plant grown under different doses and forms of selenium. *J. Plant Nutr.* **2014**, *36*, 1344–1354. [CrossRef]
69. Narváez-Ortiz, W.A.; Martínez-Hernández, M.; Fuentes-Lara, L.O.; Benavides-Mendoza, A.; Valenzuela-García, J.R.; González-Fuentes, J.A. Effect of selenium application on mineral marco- and micronutrients and antioxidant status in strawberries. *J. Appl. Bot. Food Qual.* **2018**, *91*, 321–331. [CrossRef]
70. Ahmed, N.; Zhang, B.; Bozdar, B.; Chachar, S.; Rai, M.; Li, J.; Li, Y.; Hayat, F.; Chachar, Z.; Tu, P. The power of magnesium: Unlocking the potential for increased yield, quality, and stress tolerance of horticultural crops. *Front. Plant Sci.* **2023**, *14*, 1285512. [CrossRef]
71. Feng, R.; Wei, C.; Tu, S.; Wu, F. Effects of Se on the uptake of essential elements in *Pteris vittata* L. *Plant Soil* **2009**, *325*, 123–132. [CrossRef]
72. Boldrin, P.F.; Faquin, V.; Ramos, S.J.; Furtini Boldrin, K.V.; Ávila, F.W.; Guimarães Guilherme, L.R. Soil and foliar application of selenium in rice biofortification. *J. Food Compos. Anal.* **2013**, *31*, 238–244. [CrossRef]
73. Djanaguiraman, M.; Devi, D.D.; Shanker, A.K.; Sheeba, J.A.; Bangarusamy, U. Selenium—An antioxidative protectant in soybean during senescence. *Plant Soil* **2005**, *272*, 77–86. [CrossRef]
74. Pilon-Smits, E.A.H.; Quinn, C.F.; Tapken, W.; Malagoli, M.; Schiavon, M. Physiological functions of beneficial elements. *Curr. Opin. Plant Biol.* **2009**, *12*, 267–274. [CrossRef]
75. Hawrylak-Nowak, B. Comparative effects of selenite and selenate on growth and selenium accumulation in lettuce plants under hydroponic conditions. *Plant Growth Regul.* **2013**, *70*, 149–157. [CrossRef]
76. Freeman, J.L.; Tamaoki, M.; Stushnoff, C.; Quinn, C.F.; Cappa, J.J.; Devonshire, J.; Fakra, S.C.; Marcus, M.A.; McGrath, S.P.; Van Hoewyk, D.; et al. Molecular mechanisms of selenium tolerance and hyperaccumulation in *Stanleya pinnata*. *Plant Physiol.* **2010**, *153*, 1630–1652. [CrossRef] [PubMed]
77. Malagoli, M.; Schiavon, M.; dall'Acqua, S.; Pilon-Smits, E.A.H. Effects of selenium biofortification on crop nutritional quality. *Front. Plant Sci.* **2015**, *6*, 280. [CrossRef] [PubMed]
78. Ismael, M.A.; Elyamine, A.M.; Zhao, Y.Y.; Moussa, M.G.; Rana, M.S.; Afzal, J.; Imran, M.; Zhao, X.H.; Hu, C.X. Can Selenium and Molybdenum Restrain Cadmium Toxicity to Pollen Grains in *Brassica napus*? *Int. J. Mol. Sci.* **2018**, *19*, 2163. [CrossRef]
79. Feng, R.; Wei, C.; Tu, S. The roles of selenium in protecting plants against abiotic stress. *Environ. Exp. Bot.* **2013**, *87*, 58–68. [CrossRef]
80. Schiavon, M.; Pilon, M.; Malagoli, M.; Pilon-Smits, E.A.H. Exploring the importance of sulfate transporters and ATP sulphurylases for selenium hyperaccumulation-a comparison of *Stanleya pinnata* and *Brassica juncea* (Brassicaceae). *Front. Plant. Sci.* **2015**, *6*, 2. [CrossRef]
81. Feng, R.W.; Wei, C.Y. Antioxidative mechanisms on selenium accumulation in *Pteris vittata* L., a potential selenium phytoremediation plant. *Plant Soil Environ.* **2012**, *58*, 105–110. [CrossRef]
82. Chao, W.; Rao, S.; Chen, Q.; Zhang, W.; Liao, Y.; Ye, J.; Cheng, S.; Yang, X.; Xu, F. Advances in Research on the Involvement of Selenium in Regulating Plant Ecosystems. *Plants* **2022**, *11*, 2712. [CrossRef]
83. Fan, S.; Wu, H.; Gong, H.; Guo, J. The salicylic acid mediates selenium-induced tolerance to drought stress in tomato plants. *Sci. Hortic.* **2022**, *300*, 111092. [CrossRef]
84. Tamaoki, M.; Freeman, J.L.; Marquès, L.; Pilon-Smits, E.A.H. New insights into the roles of ethylene and jasmonic acid in the acquisition of selenium resistance in plants. *Plant Signal. Behav.* **2008**, *3*, 865–867. [CrossRef]
85. Mao, X.; Hua, C.; Yang, L.; Zhang, Y.; Sun, Z.; Li, L.; Li, T. The effects of selenium on wheat fusarium head blight and DON accumulation were selenium compound-dependent. *Toxins* **2020**, *12*, 573. [CrossRef] [PubMed]
86. Troni, E.; Beccari, G.; D'Amato, R.; Tini, F.; Baldo, D.; Senatore, M.T.; Beone, G.M.; Fontanella, M.C.; Prodi, A.; Businelli, D.; et al. In Vitro Evaluation of the Inhibitory Activity of Different Selenium Chemical Forms on the Growth of a *Fusarium proliferatum* Strain Isolated from Rice Seedlings. *Plants* **2021**, *10*, 1725. [CrossRef] [PubMed]
87. Li, Q.; Xian, L.; Yuan, L.; Lin, Z.; Chen, X.; Wang, J.; Li, T. The use of selenium for controlling plant fungal diseases and insect pests. *Front. Plant Sci.* **2023**, *14*, 1102594. [CrossRef]
88. Hanson, B.; Garifullina, G.F.; Lindblom, S.D.; Wangeline, A.; Ackley, A.; Kramer, K.; Norton, A.P.; Lawrence, C.B.; Pilon-Smits, E.A.H. Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. *New Phytol.* **2003**, *159*, 461–469. [CrossRef] [PubMed]
89. Cheng, Q.; Jia, W.; Hu, C.; Shi, G.; Yang, D.; Cai, M.; Zhan, T.; Tang, Y.; Zhou, Y.; Sun, X.; et al. Enhancement and improvement of selenium in soil to the resistance of rape stem against *Sclerotinia sclerotiorum* and the inhibition of dissolved organic matter derived from rape straw on mycelium. *Environ. Pollut.* **2020**, *265*, 114827. [CrossRef] [PubMed]
90. Chen, Z.; Sun, H.; Hu, T.; Wang, Z.; Wu, W.; Liang, Y.; Guo, Y. Sunflower resistance against *Sclerotinia sclerotiorum* is potentiated by selenium through regulation of redox homeostasis and hormones signaling pathways. *Environ. Sci. Pollut. Res.* **2022**, *29*, 38097–38109. [CrossRef]

91. Companioni, B.; Medrano, J.; Torres, J.A.; Flores, A.; Rodríguez, E.; Benavides, A. Protective action of sodium selenite against fusarium wilt in tomato: Total protein contents, levels of phenolic compounds and changes in antioxidant potential. *ISHS Acta Hortic.* **2012**, *947*, 321–327. [CrossRef]
92. Somalraju, A.; Mccallum, J.L.; Main, D.; Peters, R.D.; Fofana, B. Foliar selenium application reduces late blight severity and incidence in potato and acts as a pathogen growth inhibitor and elicitor of induced plant defence. *Can. J. Plant Pathol.* **2021**, *44*, 39–55. [CrossRef]
93. Wu, Z.-L.; Yin, X.-B.; Lin, Z.-Q.; Bañuelos, G.S.; Yuan, L.-X.; Liu, Y.; Li, M. Ihibitory effect of selenium against *Penicillium expansum* and its possible mechanisms of action. *Curr. Microbiol.* **2014**, *69*, 192–201. [CrossRef]
94. Zohri, A.; Saber, S.M.; Mostafa, M.E. Effect of selenite and tellurite on the morphological growth and toxin production of *Aspergillus parasiticus* var. *globosus* IMI 120920. *Mycopathologia* **1997**, *139*, 51–57. [CrossRef]
95. Nowruzi, B.; Jalil, B.S.; Metcalf, J.S. Antifungal screening of selenium nanoparticles biosynthesized by microcystin-producing *Desmonostoc alborizicum*. *BMC Biotechnol.* **2023**, *23*, 41. [CrossRef] [PubMed]
96. Moulick, D.; Mukherjee, A.; Das, A.; Roy, A.; Majumdar, A.; Dhar, A.; Pattanaik, B.K.; Chowardhara, B.; Ghosh, D.; Upadhyay, M.K.; et al. Selenium—An environmentally friendly micronutrient in agroecosystem in the modern era: An overview of 50-year findings. *Ecotoxicol. Environ. Saf.* **2024**, *270*, 115832. [CrossRef] [PubMed]